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A NEW SPECIES OF *DIPLOPERLA* NEEDHAM AND CLAASSEN (PLECOPTERA: PERLODIDAE) FROM NORTH CAROLINA AND VIRGINIA

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ABSTRACT

A new species, *Diploperla janeae* Kondratieff & Verdone, 2017, is described from the Appalachian Mountains of Virginia and North Carolina. The new species is proposed based on details of the epiproct, lateral stylets, and general body coloration. Supporting data includes scanning electron micrographs and color images.

Keywords: Plecoptera, Perlodidae, *Diploperla janeae* Kondratieff and Verdone, 2017, Appalachian Mountains

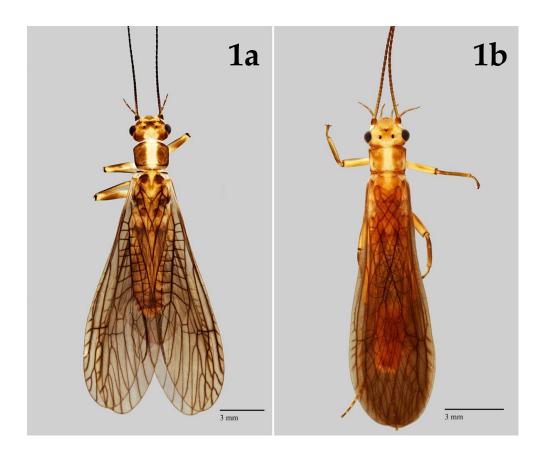
INTRODUCTION

The eastern Nearctic genus Diploperla Needham & Claassen, 1925 currently includes four species, D. duplicata (Banks, 1920), D. robusta Stark & Gaufin, 1974, *D. morgani* Kondratieff & Voshell, 1979, and *D.* kanawholensis Kirchner & Kondratieff, (Kondratieff 2004, DeWalt et al. 2017). The adult taxonomy of these species is relatively well-known (Stark & Gaufin 1974, Kondratieff & Voshell 1979, Kirchner & Kondratieff 1984). Additionally, the nymphs of the four described species are known and keyed (Kondratieff et al. 1981, Kirchner and Kondratieff 1984, Stark 2017). Recently, while collecting in the Appalachian Mountains of southwestern Virginia and western North Carolina, a new species closely related to D. duplicata was discovered. Herein, we provide a description of this new species supported by scanning electron micrographs and color images.

MATERIALS AND METHODS

Specimens of this new species were collected using a beating sheet (BioQuip, Rancho Dominguez, California, catalog # 2840C) or handpicked from substrates using forceps. Male and female specimens were kept alive in modified ventilated plastic jars (BioQuip, Rancho Dominguez, California, catalog # 8903) for 1–3 days and allowed to fully sclerotize and mate. Unfortunately, no mature eggs were available from all examined material. Live male specimens were prepared under a dissecting stereomicroscope. Using wide tipped forceps, the abdomen was gently squeezed until the lateral stylets were fully everted, then the specimen was fixed in position by briefly submerging it in near boiling water for 2–3 seconds.

Color images were captured using a Canon EOS 5D digital camera with a Canon MP-E 65 mm 5X



Figs. 1a–b. Adult habitus of *Diploperla janeae* sp. n., Helton Creek, Grayson Co., Virginia and *D. duplicata*, Laurel Fork, Floyd Co., Virginia, 1.5X. Fig. 1a. *D. janeae* sp. n, habitus, dorsal. Fig. 1b. *D. duplicata*, habitus, dorsal.

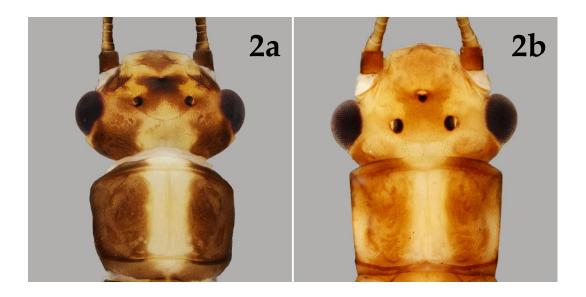
macro lens. Images are a compilation of 12–52 serial photomicrographs taken at progressively deeper focal planes using Stack Shot and controlled by Visionary Digital Passport software. Composite images were made using Zerene Stacker version 1.04. Measurements and image adjustments including background color correction, levels and sharpening functions were accomplished using Adobe Photoshop CS6 Extended.

Male terminalia were prepared for scanning electron microscopy by removing the abdomens using size 5S forceps with offset tips. Abdomens were cleaned using a fine tipped paint brush and sonicated in glass microvials filled with 80% ethanol for 30 seconds. Terminalia were serially in ethanol in ten minute intervals at concentrations of 80%, 90% and 95%. Further dehydration was

accomplished using critical point drying. Specimens were mounted on aluminum stubs using double stick copper tape and Isopropanol graphite paint. Abdomens were sputter coated in 12 nm gold in preparation for scanning electron microscopy. Micrographs of the male terminalia were obtained using a JEOL JSM-6500F Field Emission Scanning Electron Microscope at the Central Instrument Facility, Imaging Laboratory, Colorado State University

(http://cif.colostate.edu/imaging-laboratory/).

Coordinate data for new material were obtained directly using Topo Maps version 1.16 for iPhone. Additional material was examined from the C. P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado (CSUIC). Coordinate data for legacy specimen records were



Figs. 2a–b. Adult head and pronotum of *Diploperla janeae* sp. n., Helton Creek, Grayson Co., Virginia and *D. duplicata*, Bull Run Mtn, Prince William Co., Virginia, 5X. Fig. 2a. *D. janeae* sp. n, head and pronotum, dorsal. Fig. 2b. *D. duplicata*, head and pronotum, dorsal.

gathered using GEOLocate v. 3.22 (http://www.museum.tulane.edu/geolocate) and are indicated by "[]". Coordinates were plotted and mapped using ArcMap, ArcGIS 10.4.1 for Desktop. Physiographic boundaries were obtained from USGS Water Resources NSDI Node.

Morphology and terminology follow Kondratieff (2004). Abbreviations for depositories of specimens are: C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Collins. Colorado (CSUIC), Clemson University Arthropod Collection, Clemson, South Carolina (CUAC), and the United States Museum of Natural History, Washington D.C. (USNM). The holotype of the new species is deposited in the USNM. Data for all specimens examined can be downloaded from a CSV file.

RESULTS AND DISCUSSION

Diploperla janeae Kondratieff and Verdone sp. n. http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:

<u>TaxonName:501063</u>

(Figs. 1a, 2a, 3, 4a, 5a, 6a, 7a) Jane's Stripetail Material Examined. Holotype 3, Virginia: Grayson Co., Helton Creek, Rte 783, at gated trail by beaver pond, N 36.62816, W 81.56684, 13 May 2017, B.C. Kondratieff, C. Verdone (USNM). Paratypes: North Carolina: Avery Co., Jones Creek, Hwy 19E & Old Hanging Rock Road, N 35.98221, W 82.01676, 10 May 2017, B.C. Kondratieff, C. Verdone, ♂ (CSUIC). Haywood Co., Cataloochee Creek, Old Cataloochee Turnpike at bridge, GSMNP, N 35.66695, W 83.07280, 25 May 2016, C. Verdone, B.C. Kondratieff, ♂ (CSUIC). **Macon Co.,** Cold Spring Creek, jct. Cold Springs Creek Road & Forest Road 711, N 35.22323, W 83.60403, 15 May 2017, B. Kondratieff, C. Verdone, 2♀ (CSUIC). Virginia: Grayson Co., Helton Creek, Rte 783, at gated trail by beaver pond, N 36.62816, W 81.56684, 12 May 2017, B.C. Kondratieff, C. Verdone, ♂ (CSUIC); Same location, 13 May 2017, B.C. Kondratieff, C. Verdone, $2 \circlearrowleft$, \mathcal{D} (CSUIC). Tazewell Co., Roaring Fork, Roaring Fork Rd., N 37.03494, W 81.47878, 27 May 2016, C. Verdone, B.C. Kondratieff, ♂ (CSUIC). Washington Co., Straight Branch, Forest Road 837, Beaver Flats, N 36.68316, W 81.64869, 8 May 2017, B.C. Kondratieff, C. Verdone, \mathcal{L} (CSUIC).

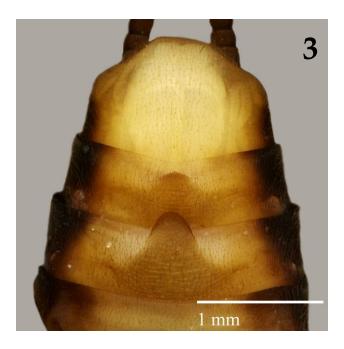


Fig. 3. Male sterna 6–10 of Diploperla janeae sp. n., Helton Creek, Grayson Co., Virginia, 5X.

Adult. Macropterous. Body length, male 11.7–13.9 mm (n = 5); female 12.1–13.3 mm (n = 4), Forewing length, male 14.8-15.5 mm (n = 5); female 13.2-15.3mm (n=4). General color yellow to light brown with dark brown to black markings (Fig. 1a). Head dorsally with a yellow "spade-shaped" spot that extends from the posterior margin of the head to beyond the epicranial suture, a dark brown to black thick "X-shaped" mark centrally that extends laterally from the anterior ocellus, and a triangular dark brown spot on the frons (Figs. 1a, 2a). Antennae dark brown to black, scape brown, yellowish tinged basally (Figs. 1a, 2a). Prothorax with wide yellow mid-dorsal stripe, slightly expanded anteriorly (Figs. 1a, 2a). Wings slightly fumose, veins dark brown (Fig. 1a). Legs yellow, femora banded with dark brown, longitudinally banded with dark brown (Fig. 1a). Mesothorax with a pair of large dark brown spots (Fig. 1a). Abdomen dorsally shaded with dark brown, yellow ventrally, laterally brown. Cerci dark brown (Fig. 1a).

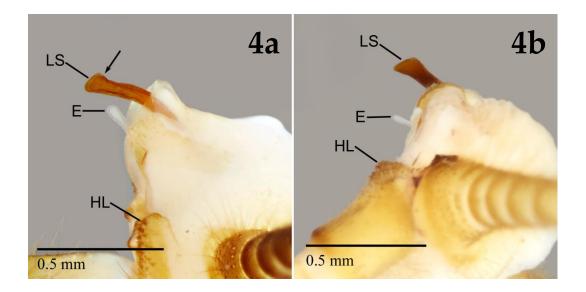
Male. Seventh and eighth abdominal sterna with well-defined lobes, lobe on seventh narrow, cone-

shaped, distal edge darkened; lobe on eighth sternum reduced, broadly rounded (Fig. 3). Tenth tergum cleft posteriorly with long hemitergal lobes studded with sensilla basiconica on the anterior surface (Fig. 4a). Lateral stylets irregularly and strongly ribbed throughout, apex expanded dorsally and ventrally, slightly asymmetrical (Figs. 4a, 6a). Epiproct short, membranous, apex bluntly rounded, without setae (Figs. 4a, 5a).

Female. Subgenital plate parabolic reaching beyond ninth sternum (Fig. 7a).

Ovum. Unknown.

Larva. Only larval exuviae of *D. janeae* were available for examination. Only one of these that could be measured had a body length of 15.5 mm (n =1). Dorsum of head with contrasting pattern; frontoclypeus moderately pigmented, brown, concolorous; anterior margin with a dense fringe of fine setae; frons moderately pigmented, brown, with a light colored, well defined M-pattern; a pair of obliquely oriented, small, pale spots lateral to median ocellus. Antennae light brown. Lacinia subtriangular, bidentate with 7 submarginal setae and no marginal setae. Pronotum dark brown;



Figs. 4a–b. Terminalia of *Diploperla janeae* sp. n., Helton Creek, Grayson Co., Virginia and *D. duplicata*, Little Rock Castle Creek, Patrick Co., Virginia, 5X. E. Epiproct, HL. Hemitergal lobe, LS. Lateral stylet. Fig. 4a. *D. janeae* sp. n, terminalia and lateral stylet, dorsal expansion (arrow), lateral. Fig. 4b. *D. duplicata*, terminalia, lateral.

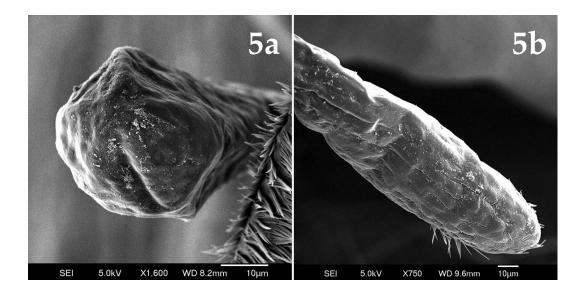
meso- and meta-nota light brown with no distinct markings. Abdomen brown with paired medial pale spots on terga 2–9. Legs lightly pigmented; femora with scattered distally directed stout setae dorsally; tibia and tarsi with a dorsal setal fringe. Cerci brown with circlets of long apical hairs on each segment.

Distribution. USA - NC, VA (Fig. 9).

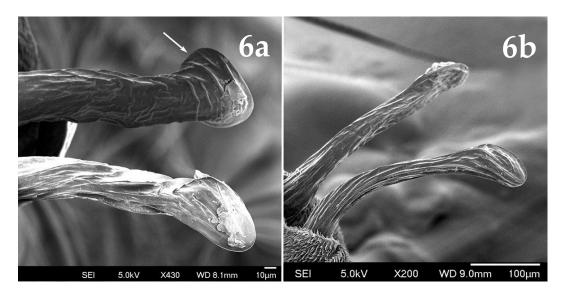
Etymology. The new species is named for Jane Earle, Mechanicsburg, Pennsylvania, in honor of her contributions to the study of North American Plecoptera. Jane has also been a loyal participant of the International Society of Plecopterologists. We propose the following common name of "Jane's Stripetail" for this species.

Diagnosis. *Diploperla janeae* is distinguished from the most similar species, *D. duplicata*, in genitalic structures, its dark coloration, and conspicuous brown to black dorsal head markings. Both species possess lateral stylets with the apices expanded. However, the apices of the new species are expanded dorsally and ventrally (Figs. 4a, 6a), whereas in *D. duplicata* the apices are expanded only ventrally (Figs. 4b, 6b). Both species are also distinguished by the apex of the epiproct. In *D.*

janeae, the epiproct apex is bluntly rounded without setae (Figs. 4a, 5a), whereas the epiproct apex of the D. duplicata is more elongate with sparse setae (Figs. 4b, 5b). The female subgenital plate of *D. janeae* is similar to D. duplicata (Figs. 7a, 7b), but the distinctive habitus coloration will serve to distinguish both taxa. Diploperla janeae is yellow to light brown with dark brown to black markings, slightly fumose wings with dark brown veins, and dark brown antennae and cerci (Fig. 1a), compared to D. duplicata which is yellow with light brown to brown markings, amber colored wings with light brown veins to light brown to brown antennae and cerci (Fig. 1b). Additionally, the head pattern of the two species differ. The head of D. janeae, has a yellow "spade-shaped" spot that extends from the posterior margin of the head to beyond the epicranial suture, and a dark brown to black thick "X-shaped" mark centrally that extends laterally from the anterior ocellus (Fig. 2a). Whereas, the head of *D. duplicata* usually has a closed light brown spot covering the interocellular area with arms that extend anteriorly from the anterior ocellus (Fig. 2b). Three other Diploperla species, D. kanawholensis,



Figs. 5a–b. Epiproct of *Diploperla janeae* sp. n., Helton Creek, Grayson Co., Virginia and *D. duplicata*, Laurel Fork, Floyd Co., Virginia. Fig. 4a. *D. janeae* sp. n, epiproct, anterior, 1600X. Fig. 5b. *D. duplicata*, epiproct, lateral, 750X.



Figs. 6a–b. Paraprocts of *Diploperla janeae* sp. n., Helton Creek, Grayson Co., Virginia and *D. duplicata*, Laurel Fork, Floyd Co., Virginia. Fig. 6a. *D. janeae* sp. n, paraprocts and dorsal expansion (arrow), lateral, 430X. Fig. 6b. *D. duplicata*, paraprocts, lateral, 200X.

D. morgani, and *D. robusta*, are darkly marked as *D. janeae*. But males are easily distinguished by the genitalic characters and the shape of the lobe of sternum 7, which is broadly rounded apically (fig.

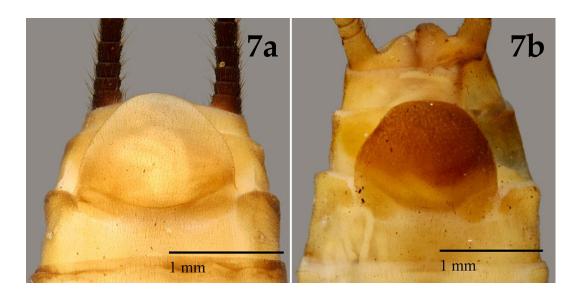
8.90, Kondratieff 2004) in *D. kanawholensis*, *D. morgani*, and *D. robusta*, and narrowly rounded apically in the new species (Fig. 3).

The following key is adapted from Kondratieff (2004). New couplets are given to include the new species.

Key to Diploperla Species Adults

- 3 Epiproct sclerotized, prominent, long and slender, in dorsal view (fig. 8.88, Kondratieff 2004); apex of lateral stylets acute (fig. 8.88, Kondratieff 2004); subgenital plate nearly parallel side, apical margin broadly rounded or truncate (fig. 8.91, Kondratieff 2004). Distribution: WV, VA, NC D. kanawholensis

Additional material examined. Diploperla duplicata: Georgia/South Carolina: Rabun Co./Oconee Co., Chattooga River, at State Rte 28, N 34.91920, W 83.16870, 5 May 2010, T. Sheeha, & (CUAC). **North** Carolina: Alleghany Co., Big Sandy Creek, Stone Mountain Road, N 36.39445, W 81.03341, 29 May 2006, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, 53, 49 (CSUIC). Caldwell Co., Johns River, Old Johns River Rd. 1.5 mi. upstream of Hwy 90 NE of Colletsville, N 35.93379, W, 81.69036, 3 May 2005, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, & (CSUIC). Guilford Co., Big Alamance Creek, Rte 1005, [N 35.98405, W 79.65727], 3 June 1981, B.C. Kondratieff, ♂ (CSUIC). **Jackson Co.,** trib. to Silver Run, at NC 107 bridge, [N 35.06170, W 83.06911], 4 May 2008, J.L. Robinson, 13 \Diamond , \Diamond (CSUIC). **Macon Co.**, Big Creek, waterfall at Buck Creek Rd., [N 35.09163, W 83.19637], 4 July 2008 (Light trap), J.L. Robinson, ♂ (CSUIC); Turtle Pond Creek, 1st bridge below US 64, [N 35.07587, W 83.25693], 3 June 2008, J.L. Robinson, & (CSUIC). Wilkes Co., Hunting Creek, Hwy 115 NW of Elkville, N 36.07879, W 80.97789, 2 May 2005, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, 60° , 39° (CSUIC). **Tennessee: Sevier Co.,** Abrams Creek, Cades Cove (GSMNP), [N 35.60484, W 83.77094], 1 June−12 June 2003, C.R. Parker, ♂ (CSUIC); Same location, 30 April-6 May 2004, C.R. Parker, 4♂ (CSUIC); Wildcat Branch, Cades Cove (GSMNP), [N 35.58133, W 83.83737], 1 June-3 June 2003, C.R. Parker, ♂ (CSUIC); Twin Creeks (Le Conte Creek), Research Station (GSMNP), [N 35.68670, W 83.50072], 14 May-21 May 2004, A.J. Major, 3d (CSUIC). Virginia: Appomattox Co., Rough Creek, Rte 627, [N 37.33592, W 78.69583], 16 April 1983, emerged 2 May 1983, B.C. Kondratieff, 3, 2, 1L, 3 exuvia (CSUIC). **Bedford Co.**, Big Otter River, Rte 221, [N 37.36462, W 79.41947], 16 April 1983, B.C. Kondratieff, \mathcal{O} , \mathcal{O} (CSUIC); lake outlet, Blue Ridge Parkway (BLRI), N 37.44439, W 79.60220, 9 August



Figs. 7a–b. Female subgenital plate of *Diploperla janeae* sp. n., Helton Creek, Grayson Co., Virginia and *D. duplicata*, Little Rock Castle Creek, Patrick Co., Virginia, 5X. 7a. *D. janeae* sp. n, female subgenital plate. 7b. *D. duplicata*, female subgenital plate.

2006, J.L. Robinson, ♂, ♀ (CSUIC); Little Stony Creek, Hwy 43, Peaks of Otter Picnic Area (BLRI), N 37.44540, W 79.59713, 17 June 2016, C. Verdone ♀ (CSUIC); [North Otter Creek], [Rte 644], [N 37.39236, W 79.45324], 22 May 1980, Light Trap, J. Despins, 26 (CSUIC). Carroll Co., Big Laurel Creek, Rte 639 (BLRI), N 36.65098, W 80.51871, 30 May 2016, C. Verdone, B.C. Kondratieff, ♀ (CSUIC); Big Reed Island Creek Rd. between Rte 640 & Rte 645, N 36.67665, W 80.51019, 30 May 2016, C. Verdone, B.C. Kondratieff, 3♀ (CSUIC); Crooked Creek, Rte 620, Trout fishing trail, N 36.67296, W 80.80853, 29 May 2016, C. Verdone, B.C. Kondratieff, 5♀ (CSUIC); North Fork Stewarts Creek, BLRI, N 366.60630, W 80.81663, 11 May 2017, B.C. Kondratieff, C. Verdone, ♀ (CSUIC); Pine Creek, nr. jct. Rte 633 & Rte 640, N 36.66773, W 80.51043, 30 May 2016, C. Verdone, B.C. Kondratieff, $3 \stackrel{?}{\circ}$, $8 \stackrel{?}{\circ}$ (CSUIC); Stewarts Creek, ca. 1 mi. W Lambsburg, [N 36.58108, W 80.76524], 29 May 1999, R.L. Hoffman, ♂ (CSUIC); Stewarts Creek, Rte 696, N 36.58083, W 80.76437, 30 May 2016, B.C. Kondratieff, C. Verdone, ♂ (CSUIC); Same location, 11 May 2017, B. C. Kondratieff, C. Verdone, 2♀ (CSUIC); trib. to Turkey Creek, Rte 620, N 36.61184, W 80.77082, 11 May 2017, B.C. Kondratieff, C.

Verdone, 2♂ (CSUIC). **Floyd Co.,** Laurel Fork, Blue Ridge Parkway, S. of Buffalo Mtn. turnoff (BLRI), N 36.77258, W 80.48511, 26 May 2016, B.C. Kondratieff, C. Verdone, 53, 69 (CSUIC); Mabry Mill, Blue Ridge Parkway, MP 176.1 (BLRI), [N 36.75014, W 80.40478], 28 May 2006, J.L. Robinson, 36 (CSUIC); spring trib., Blue Ridge Parkway, Spring house S. of Rte 642 (BLRI), N 37.01133, W 80.12679, 31 May 2016, B.C. Kondratieff, C. Verdone, \bigcirc (CSUIC); trib. to Dodd Creek, Blue Ridge Parkway (BLRI), N 36.86353, W 80.28702, 31 May 2016, B.C. Kondratieff, C. Verdone, $2 \circlearrowleft$, $4 \circlearrowleft$ (CSUIC); trib. to Little River, Blue Ridge Parkway, N of Rte 642 "Poff" (BLRI), N 37.01437, W 80.12343, 19 May 2017, C. Verdone, B.C. Kondratieff, S. Roble, $3 \stackrel{?}{\circ}$, $3 \stackrel{?}{\circ}$ (CSUIC). Franklin Co., Grassy Fork, Co. Rd. 613, 2 mi. SE Snow Creek, [N 36.81093, W 79.74514], 6 May 2000, R.L. Hoffman, 4♂ (CSUIC); Same location, 21 May 2002, B.C. Kondratieff, R.F. Kirchner, ♂, 2♀ (CSUIC). Same location, 14 May 2003, O. Flint, R.L. Hoffman, 23 (CSUIC). Giles Co., Stony Creek, Rte 635, nr. Rte 628, [N 37.39125, W 80.66073], 13 May 1981, B.C. Kondratieff, 1L (CSUIC). Grayson Co., East Fork Chestnut Creek, Blue Ridge Parkway (BLRI), N 36.57045, W 80.85896, 25 May 2017, C. Verdone, ♂

(CSUIC). Henry Co., Machine Creek, Rte 619, N Leatherwood, [N 36.80045, W 79.73924], 3 May 2008, O. Flint Jr., & (CSUIC). Madison Co., Robinson River, Rte 600, N 38.52717, W 78.35015, 1 June 2016, C. Verdone, ♂ (CSUIC); Same location, 2 June 2016, C. Verdone, B. C. Kondratieff, S. Roble, ♂ (CSUIC). Montgomery Co., Mill Creek, Rte 785, [N 37.26133, W 80.34061], 12 April 1979, PTS, 1L (CSUIC). Patrick Co., Dan River, Rte 648, N 36.62230, W 80.44497, 11 May 2017, C. Verdone, B.C. Kondratieff, 23, 29(CSUIC); Hales Creek, above water plant, Fairy Stone State Park, [N 37.17100, W 79.66596], 9 June 2002, R.L. Hoffman, 2♂ (CSUIC); Little Rock Castle Creek, Rte 605, off Rte 8, (BLRI), [N 36.80783, W 80.33112], 13 May 1979, B.C. Kondratieff, ♀ (CSUIC); Same location, 26 May 2016, C. Verdone, B.C. Kondratieff, ♂ (CSUIC); Same location, 9 May 2017, B.C. Kondratieff, C. Verdone, 2♀ (CSUIC); Mayberry Creek, Blue Ridge Parkway, MP 180.7 (BLRI), [N 36.70842, W 80.44395], 8 June 2007, C. Parker, 6♂ (CSUIC); Mayberry Creek, Blue Ridge Parkway (BLRI), N 36.70842, W 80.44395, 26 May 2016, B.C. Kondratieff, C. Verdone, $5 \circlearrowleft$, \circlearrowleft (CSUIC); Rock Castle Creek, end of Rte 605, [N 36.80712, W 80.33108] 3 April 1976, J. Harrison, 1L (CSUIC); Same location, 11 April 1976, R. Paul, W. Ruska, 1L (CSUIC); Same location, 21 June 1996, R.L. Hoffman, 3♂ (CSUIC). trib. to Rock Castle Creek, Rte 605 at cemetery, N 36.80874, W 80.32550, 26 May 2016, B.C. Kondratieff, C. Verdone, \mathcal{Q} (CSUIC); Same location, 9 May 2017, B.C. Kondratieff, C. Verdone, ♀ (CSUIC). Pittsylvania Co., Allen Creek, Rte 40, [N 36.95393, W 79.14423], 26 April 1979, emerged 9 May 1979, B.C. Kondratieff, ♀, 1L (CSUIC). Prince William Co., Bull Run Mtn., Jackson Hollow Campground, N 38.876667, W 77.69000, 28 May-10 June 2011 (malaise trap), D.R. Smith, 43, 29(CSUIC); Same location, 26 May-11 June 2012 (malaise trap), D.R. Smith, 33, 14 (CSUIC); Same location, 12 June-27 June 2012 (malaise trap), D.R. Smith, 2^{\land} , \mathcal{D} (CSUIC). Same location, 23 May–6 June 2013 (malaise trap) D.R. Smith, $2 \circlearrowleft$, \circlearrowleft (CSUIC); Same location, 30 May-15 June 2015 (malaise trap), D.R. Smith, 2d (CSUIC). **Tazewell Co.**, East Fork Cove Creek, Rte 662, [N 37.19459, W 81.30070], 12 June 1983, B.C. Kondratieff, ♀ (CSUIC). **West Virginia: Nicholas Co.,** [Jims Branch], 6 mi. W of Richwood, [N 38.22976, W 80.66532], 29 April 1983, B.C. Kondratieff, R.F. Kirchner, ♂ (CSUIC).

Diploperla kanawholensis: North Carolina: Caldwell Co., Johns River, Old Johns River Rd. NE of Colletsville, N 35.94711, W 81.70472, 2 May 2015, B.C. Kondratieff, R.F. Kirchner, R.E. Zuellig, D.R. Lenat, ♂ (CSUIC); Yadkin River, Grandin Rd. N Grandin, [N 36.05797, W 81.43175], 18 April 2007, B.C. Kondratieff, R.F. Kirchner, D.R. Lenat, 2, 2 exuvia (CSUIC). Wilkes Co., trib. to Silver Run, at NC 107 bridge, [N 35.06170, W 83.06911], 4 May 2008, J.L. Robinson, ♀ (CSUIC). Virginia: Bedford Co., Big Otter River, Rte 460, [N 37.30852, W 79.39388], 9 February 1983, emerged 19 March 1983, B.C. Kondratieff, ♀ (CSUIC); Big Otter River, Rte 221, [N 37.36455, W 79.41951], 22 March 1983, B.C. Kondratieff, 3L (CSUIC); Same location, 22 April 1983, ♂ (CSUIC); North Otter Creek, Rte 122, jct. Rte 639, [N 37.46016, W 79.46469], 11 May 1981, B.C. Kondratieff, 2 (CSUIC). West Virginia: Braxton/Lewis Co., Little Kanawha River, 1mi. SW of Wildcat, [N 38.74376, W 80.45531], 29 April 1983, R.F. Kirchner, B.C. Kondratieff, & (CSUIC, paratype); Same data as previous, 2♂, 2♀, 2L (CSUIC, paratypes).

Diploperla morgani: Virginia: Bland Co., Wolf Creek, Grapefield Rd., Wolf Creek Picnic Area, N 37.18026, W 81.19496, 8 June 2016, C. Verdone, B.C. Kondratieff, \Diamond , \Diamond (CSUIC); Same location, 12 June 2016, C. Verdone, ♀ (CSUIC); Same location, 7 May 2017 C. Verdone, B. Kondratieff, S. Roble, & (CSUIC); Wolf Creek, Rte 614/Grapefield Rd., Stephen Levitt Property, N 37.14702, W 81.26314, 4 June 2016, C. Verdone, B.C. Kondratieff, 3, 4(CSUIC); Same location, 6 June 2016, C. Verdone, B.C. Kondratieff, $3 \circlearrowleft$, $3 \updownarrow$ (CSUIC); Same location, 8 June 2016, C. Verdone, B.C. Kondratieff, 23, 29(CSUIC); Same location, 23 May 2017, C. Verdone, 3, 3 (CSUIC); Same location, 27 May 2017, C. Verdone, D. Fuller, ♀ (CSUIC). Scott Co., Copper Creek, jct. Rte 611 & 612, N 36.75322, W 82.37894, 8 May 2017, C. Verdone, B.C. Kondratieff, \mathcal{L} (CSUIC). Montgomery Co., Mill Creek, Rte 785,



Fig. 8. Type locality. Helton Creek, Grayson Co., Virginia, USA.

[N 37.26129, W 80.34056], 11 April 1978, G. D. Hutton, 2L (CSUIC); Same location, 15 April 1980, emerged 28 April 1980, B.C. Kondratieff, ♀ (CSCU); Same location, 25 April 1980, B.C. Kondratieff, 3, 2(CSCU); Same location, 14 April 1981, emerged 26 April 1981, B.C. Kondratieff, 2♀ (CSUIC). Smyth Co., North Fork Holston River, Rte 620 nr. jct. Rte 716, [N 36.94807, W 81.49062], 4 April 1981, emerged 28 April 1981, B.C. Kondratieff, 3, 2 (CSUIC); Same data as previous, emerged 30 April 1981, B.C. Kondratieff, 3, 2 (CSUIC); North Fork Holston River, Rte 42 nr. jct. Rte 633, [N 36.91345, W 81.70903], 9 May 1981, B.C. Kondratieff, ♂ (CSUIC); North Fork Holston River, Rte 716 nr. Rte 620[N 36.94807, W 81.49062], 4 April 1982, emerged 22 April 1982, B.C. Kondratieff, 3, 2 (CSCU). Washington Co., Logan Creek, Rte 80, 0.25 mi. SE of North Fork Holston River, N 36.81713, W 81.92087, 4 May 2017, C. Verdone, B.C. Kondratieff, ♀ (CSUIC).

Diploperla robusta: Kentucky: Edmonson Co., Cub Creek, KY 70, E of Roundhill, [N 37.24202, W 86.38722], 11 May 2000, B.C. Kondratieff, 3 (CSUIC). Floyd Co., Shade Branch, Jenny Wiley Dr., Jenny Wiley State Park, [N 37.68706, W 82.70115], 13 May 2000, B.C. Kondratieff, R.F. Kirchner, ♂ (CSUIC). Madison Co., Cowbell Creek, Berea College Forest, [N 37.54404, W 84.20994], 20–27 May 2013, Chapman, Stedfeld, & (CSUIC). Rowan Co., Logan Hollow of North Fork Triplett Creek, 3 mi. W Moorehead, [N 38.19101, W 83.49124], 25 April 1987, B.C. Kondratieff, $2 \circlearrowleft$, \circlearrowleft , 18L (CSUIC); Same location, 11 May 1988, B.C. Kondratieff, 23, 39 (CSUIC); Same location, 15 May 1990, B.C. Kondratieff, R.F. Kirchner, J. Welch, 43, 59 (CSUIC); Same location, 24 May 1992, B.C. Kondratieff, R.F. Kirchner, $2 \circlearrowleft$, $4 \circlearrowleft$ (CSUIC); Ramey Creek, Cave Run Lake, Daniel Boone National Forest, 1 mi. N of Rte 801, [N 38.10426, W 83.45037], 28 April 1987, B.C. Kondratieff, 5L (CSUIC); Same location, 1 June 1992,

B.C. Kondratieff, R.F. Kirchner, & (CSUIC). Ohio: Lawrence Co., Storms Creek, Wayne National Forest, Lake Vesuvius, [N 38.63213, W 82.59582], 19 May 1993, B.C. Kondratieff, R.F. Kirchner, $3 \stackrel{?}{\circ}$, $2 \stackrel{?}{\circ}$ (CSUIC). Scioto, Co., Pond Lick Run, jct. Turkey Creek, Hwy 125, [N 38.70817, W 83.13545], 10 May 1988, B.C. Kondratieff, ♂ (CSUIC); Turkey Creek, jct. Pond Lick Run, Hwy 125, [N 38.70814, W 83.13515], 19 May 1990, B.C. Kondratieff, R.F. Kirchner, J. Welch, ♀ (CSUIC); Wayne Co., Agriculture experimental station arboretum, [N 40.78345, W 81.91738], 18 May 1960, George M. Kelly, ♀ (CSUIC). **Pennsylvania: Clarion Co.,** Toms Run, upstream mouth Browns Run, Cook Forest State Park, N 41.36189, W 79.23451, 17 June 2009, S. Harris, ♀ (CSUIC). Erie Co., Sixmile Creek, Hazel & Burt Morgan, N 42.11944, W 79.95416, 25 May 1978, E.C. Masteller, 4^{\wedge} (CSUIC); Same location, 27 May 1979, E.C. Masteller, $4 \circlearrowleft$, $4 \circlearrowleft$ (CSUIC). **Tennessee:** Bledsoe Co., [Soddy Creek], Jimmy Johnson Rd. (Rte 305), N of TN 111, 0.3 mi. N of Sequatchie Co. line, N 35.38593, W 85.26441, 6 May 1998, C.R. Parker, D.A. Etnier, ♀ (CSUIC). Cumberland Co., Pine Creek Headwaters, Catoosa WMA, N 36.11500, W 84.97300, 7 June 2016, S.M. Roble, ♀ (CSUIC); Pine Creek Headwaters, Catoosa WMA, N 36.11578, W 84.97250, 11 June 2016, S.M. Roble, 2♀ (CSUIC); [North Fork Elmore Creek], 3 mi. N of Adams Bridge Obed River, N 36.10321, W 84.94164, 13 May 1988, B.C. Kondratieff, R.F. Kirchner, 3, 3(CSUIC); Same location, 22 May 1994, B.C. Kondratieff, R.F. Kirchner, \Diamond , \Diamond (CSUIC) **Franklin** Co., Unnamed stream, off TN Rte 16, N 35.08046, W 86.04062, 16 May 2017, S.M. Roble, 3♂, ♀ (CSUIC); Unnamed stream, off TN Rte 16, N 35.07464, W 86.03960, 17 May 2017, S.M. Roble, ♀ (CSUIC). Virginia: Craig Co., Johns Creek, Rte 632, [N 37.41064, W 80.39792], 21 April 1980, emerged 6 May 1980, B.C. Kondratieff, \Diamond , \Diamond (CSUIC). Montgomery Co., Craig Creek, Rte 621, [N 37.29093, W 80.45168], 21 April 1980, Bishop, ♂ (CSUIC); Craig Creek, Rte 621, 0.5 mi. off Rte 460, [N 37.29093, W 80.45168], 5 June 1979, B.C. Kondratieff, ♂ (CSUIC); Flatwood Branch, Rte 603, [N 37.23777, W 80.2688], 30 April 1978, emerged 5 May 1978, B.C.

Kondratieff, ♂ (CSUIC); Small spring-fed stream entering Craig Creek, Rte 621, 3 mi. off Rte 460, [N 37.29070, W 80.45221], 22 May 1980, B.C. Kondratieff, 3, 3 (CSUIC). Same location, 26 May 1980, B.C. Kondratieff, ♀ (CSUIC); Same location, 28 May 1980, B.C. Kondratieff, 3, 3 (CSUIC); Same location, 28 May 2016, B.C. Kondratieff, C. Verdone, ♀ (CSUIC). **Scott Co.,** Laurel Branch, FR 700, 1km from FR 701, N 36.87556, W 82.48100, 6 May 2017, C. Verdone, B. Kondratieff, S.M. Roble, ♂ (CSUIC). Washington Co., trib. to North Fork Holston River, Wootan Gap, Rte 802, N 36.74332, W 82.22892, 8 May 2017, C. Verdone, B.C. Kondratieff, \mathcal{L} (CSUIC). West Virginia: Cabell Co., Fourpole Creek, Huntington, Rte 10, N 38.39227, W 82.40340, 28 April 1994, R.F. Kirchner, A.P. Marchesa, 23, 1L (CSUIC). Nicholas Co., [Jims Branch], 6 mi. W of Richwood, [N 38.22976, W 80.66532], 29 April 1983, B.C. Kondratieff, R.F. Kirchner, \Diamond , \Diamond , \Diamond (CSUIC); North Fork Cherry River, Hwy 55/39, 3 mi. NE Richwood, [N 38.25450, W 80.51189], 17 May 1993, B.C. Kondratieff, R.F. Kirchner, & (CSUIC). Pocahontas Co., East Fork Greenbrier River, Island Campground, N 38.58039, W 79.70449, 16 May 1990, B.C. Kondratieff, R.F. Kirchner, J. Welch, 2♀ (CSUIC); Left Branch, above jct. North Fork Cherry River, Rte 39/55, [N 38.19071, W 80.35078], 17 May 1993, B.C. Kondratieff, R.F. Kirchner, ♂, 7♀, 8L (CSUIC); North Fork Cherry River, jct. Left Branch, Hwy 39/55, [N 38.19048, W 80.35074], 17 May 1993, B. C. Kondratieff, R. F. Kirchner, 3, 2 (CSUIC); North Fork Cherry River, 1 mi. NW Falls of Hills Creek, WV 39, [N 38.18780, W 80.34724], 2 June 1992, B.C. Kondratieff, & (CSUIC); Hills Creek, Hills Creek Scenic Area, [N 38.18557, W 80.31634], 15 May 1990, B.C. Kondratieff, R.F. Kirchner, $4 \circlearrowleft$, $2 \circlearrowleft$ (CSUIC).

Biological notes. There is no information about the biology or life cycle of *D. janeae*. Based on the available records, the emergence period appears to be from early to late May. The new species has been collected from 1st to 4th order streams in the Blue Ridge, and Ridge and Valley physiographic provinces from southwestern Virginia to southwestern North Carolina. The type locality,

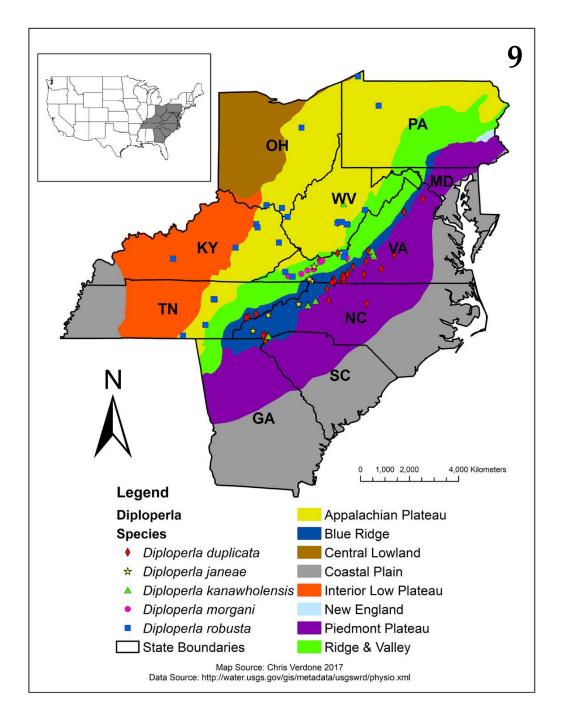


Fig. 9. Distribution map of examined material and physiographic provinces. See text for comprehensive list of states occupied for some species.

Helton Creek drains the southwest side of Mount Rogers, the highest mountain in Virginia at 1,746 m (5,729 ft.), and has a diverse stonefly fauna. Other adult stoneflies collected with the new species at the type locality were *Alloperla concolor* Ricker, 1936, *Amphinemura wui* (Claassen, 1936), *Cultus verticalis* (Banks, 1920), *Haploperla parkeri* Kirchner & Kondratieff, 2005, *Isoperla cotta* Ricker, 1952, *I.*

dewalti Verdone & Kondratieff, 2017, I. reesi Szczytko & Kondratieff, 2015, Leuctra duplicata Claassen, 1923, L. ferruginea (Walker, 1852), L. grandis Banks, 1906, L. sibleyi Claassen, 1923, Malirekus hastatus (Banks, 1920), Ostrocerca albidipennis (Walker, 1852), Paraleuctra sara (Claassen, 1937), Pteronarcys scotti Ricker, 1952, Sweltsa lateralis (Banks, 1911), S. mediana (Banks, 1911), Tallaperla anna (Needham & Smith, 1916), T. maiyae Kondratieff, Kirchner & Zuellig, 2007, and T. maria (Needham & Smith, 1916).

Discussion. Kondratieff (2004) provided an illustration of the lateral stylets of *D. duplicata* (fig. 8.84) from a specimen collected from Big Otter River, Bedford County, Virginia, which was not accurately illustrated. No specimens of *D. duplicata* examined in this study, including the specimen from Big Otter River, have lateral stylet apices with a dorsal expansion projecting markedly beyond the basal plane. In several male specimens of *D. duplicata* from North Carolina, the head pattern is similar to the new species, but the lateral stylets clearly lack the characteristic dorsal expansion of *D. janeae*.

ACKNOWLEDGEMENTS

Kim Vanderpool, Colorado State University, Department of Biomedical Sciences is thanked for preparing the SEM images. We thank Steven Beaty, North Carolina Department of Environmental Quality, Division of Water Resources, Raleigh, North Carolina for providing larval specimens of *Diploperla* and other information. We especially thank our colleagues who joined the authors on numerous productive trips, especially the late David R. Lenat, R.F. Kirchner, (Huntington, West Virginia), Dr. Robert E. Zuellig (USGS, Fort Collins, Colorado), and Dr. Steve M. Roble (Richmond, Virginia).

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Submitted 1 November 2017, Accepted 22 November 2017, Published 6 December 2017

Hosted and published at the University of Illinois, Illinois Natural History Survey, Champaign, Illinois, U.S.A.